Dynamics of antioxidant enzyme activities during the senescence of flag leaf in wheat plants under drought stress

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Physiological senescence of flag leaf, which plays a major role in the absorption of solar energy during photosynthesis in wheat and maintaining plant productivity, is one of the most important parameters for ensuring high productivity under stress conditions. The dynamics of the activity of ascorbate peroxidase (APO) and catalase (CAT) enzymes were investigated under drought stress and normal watering conditions in wheat genotypes with contrasting tolerance during the physiological senescence of the flag leaf. Measurements were made at 6 points, 7 days after the formation of flag leaves. It was discovered that the activity of enzymes varied according to the natural and stress-induced senescence of the leaf. The activity of CAT was relatively low in the youngest leaves and in the last stage of senescence in both watered and drought-exposed plants. The maximum activity of CAT was observed in 21-day-old flag leaves of control plants, in 28-day-old leaves of the tolerant Vugar genotype exposed to drought, and in 21-day-old leaves of the sensitive Tartar genotype. APO activity was higher in young leaves. Although the activity of this enzyme in the control plants was increasing at the end of senescence again, a sharp decline was recorded in plants exposed to stress due to the rapid senescence. Unlike the sensitive genotype, APO activity increased proportionally with leaf age in the tolerant genotype.

Keywords: *Triticum durum.* Desf., *drought, flag leaf senescence, ascorbate peroxidase, catalase*

INTRODUCTION

Wheat is one of the most productive plants among cereals and the main source of plant-based protein in the human diet. Global warming is a serious threat to the biodiversity of the planet. Consequently, warming of the air can give rise to aggravation of stress factors such as drought, reduced productivity, loss of sustainability in agricultural production, the extinction of a number of valuable species, and the risk of food shortages worldwide. The study of the senescence of the flag leaf, which plays an essential role in absorbing solar energy during photosynthesis in wheat and determines the productivity of the plant, is one of the most important parameters for ensuring high productivity under stress conditions. The leaves that perform photosynthesis correlate with grain yield. The top three leaves in wheat, especially the flag leaf, have been identified as the primary source of photoassimilates collected in the seed. In wheat genotypes, flag leaves provide higher productivity by performing longer photosynthesis in comparison to other leaves. Senescence of the flag leaf is connected to the period of redistribution of resources from the source to the acceptor in the grain filling cycle. Initiation and velocity of senescence are essential factors in determining the productivity potential because approximately 30-50% of assimilates are provided by photosynthesis of flag leaf during the grain filling process in wheat (Verma, 2003). Leaf senescence is an agronomic feature influenced by abiotic and biotic stresses that affect plant productivity and product quality. The first sign of leaf senescence is chlorophyll breakdown and the reduction in photosynthesis (Saeedipour and Moradi, 2010). Changes occur in metabolism in leaf cells during senescence. Leaf senescence consists of consecutive stages: catabolism of chlorophyll, proteins, lipids and nucleic acids associated with the transfer of nutrients to the developing seeds, and ultimate

plant death. Senescence is related to the increase in the amount of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O), superoxide, and hydroxyl radicals with more toxic effects (Van Breusegem and Dat, 2006). In turn, when the toxic ROS reach a certain level, they oxidize proteins, lipids and DNA. This results in peroxidation of the lipids, cell damage and death due to the weakened antioxidant status of the leaf. (Kukavica and Jovanovic, 2004). In plant cells, ROS occur continuously as a by-product of metabolic pathways localized in different cell compartments. Chloroplasts and mitochondria dominate among them because of their strong oxidizing properties or rapid electron flow (Apel and Hirt, 2004; Miller et al., 2008). Under stable physiological conditions, ROS are removed through various antioxidant protection components that are restricted with a certain compartment. Although there are several enzyme mechanisms in the chloroplast and mitochondria to remove ROS, CAT and APO are involved in their most important reactions. Because the balance can be broken between the formation and removal of ROS during senescence, an important increase in ROS is likely to affect chloroplasts with strong photooxidative potential. Processes involving ROS are under the control of chloroplasts. The main aim of the current research was to analyze the dynamics of changes in APO and CAT activity, which are key components of antioxidant defense systems in the process of natural as well as drought-induced senescence of the flag leaf of wheat genotypes.

MATERIALS AND METHODS

As a research object, durum wheat (*Triticum durum* Desf.) genotypes, Vugar (tolerant), and Tartar (sensitive) were taken from the gene pool of the Research Institute of Crop Husbandry. Plants were grown in the field over a wide area under normal water supply and drought conditions.

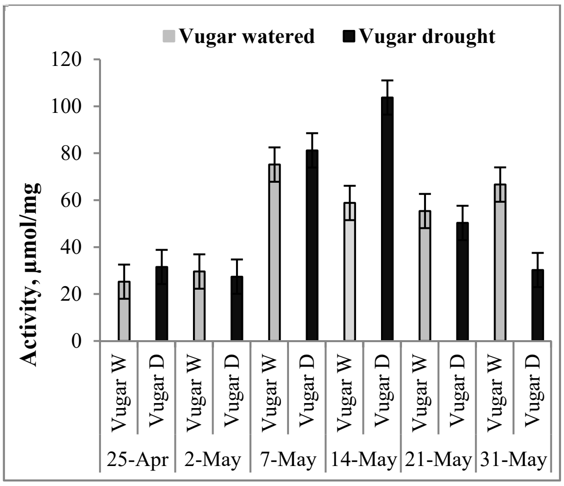
Determination of ascorbate-peroxidase activity: To determine APO activity, 1 g of leaves were taken and crushed in 10 ml of 50 mM potassium-phosphate buffer (pH 7.6) in cold. After adding 0.3 g PVP, it was filtered and centrifuged at a frequency of 12,000g, for 10 minutes. The reaction mixture consists of 50 µM 0.1 mM H_2O_2, 2.55 ml 50 mM phosphate buffer (pH 7.6) and 300 µl of the plant extract obtained from centrifugation of the homogenate. The optical density was measured on a ULTROSPEC 3300 PRO ("AMERSHAM", USA) spectrophotometer in the wavelength of 290 nm, using a non-enzyme extract as a control. As the APO activity measure, reduced optical density was taken within the first 30 seconds of the reaction and was calculated in the units of µmol / mg protein minute taking account $\varepsilon = 2{,}8\ mM^{-1}\ sm^{-1}$ (Nakano and Asada, 1981) as a coefficient of molar extinction.

Determination of catalase activity: To determine the activity of CAT, 1 g of leaf tissues was crushed in 10 ml of 50 mM calcium-phosphate buffer (pH 7.0). The homogenate was filtered and centrifuged at a frequency of 8,000 g, for 10 minutes. 25 µl of the enzyme extract was poured on 2.9 ml of the phosphate buffer (pH 7.0). 90 µl of 3% H_2O_2 was added to this solution just before the measurement. Optical density drop was measured at 240 nm, for 1 min using the spectrophotometer. The activity of the enzyme was calculated on the basis of molar extinction coefficient $\varepsilon = 39{,}4\ mM^{-1}\ sm^{-1}$ (Kumar and Knowles, 1993) in units of µmol/mg protein per minute.

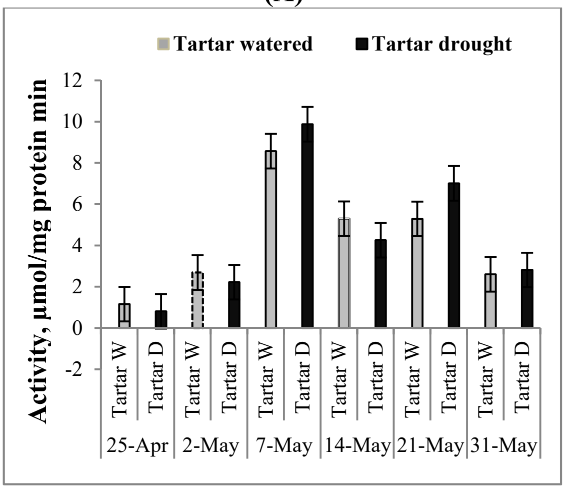
RESULTS AND DISCUSSION

Although catalase is localized in peroxisomes and glyoxysomes, its specific isoform is also observed in plant mitochondria and chloroplasts (Menshikova and Zenkov, 1993). Increased activity of catalase contributes to plant ~~for~~ better protection from the effects of oxidative stress and provide improvement to survivability in response to adverse environmental impacts (Nicholls et al., 2001). There have been significant changes in catalase activity during leaf senescence due to the effects of drought stress. In our studies, the enzyme activity was maintained at high levels in the tolerant genotype by the end of the vegetation period, and in the unstable genotype, it was steadily declining. Catalase activity was also minimal in stress-exposed plants during the early vegetation period and increased as the leaf aged. The maximum activity under drought stress in the tolerant genotype leaves was observed at 81±2

μmol / mg protein minute (May 14), and as the leaf age increased, this showed a minimum value of 30±2 μmol/mg protein minute on the 42nd day (May 31).



(A)



(B)

Fig. 1. Alterations of the CAT enzyme activity during leaf senescence in drought stress-exposed wheat plants (A-Vugar and B-Tartar).

Two peaks were also recorded in the activity of catalase in the sensitive variety. The first peak indicating the maximum activity was observed on the 21st day (May 7), the second peak on the 35th day (May 21). Although it showed the minimum activity at the end of senescence, the activity was slightly higher compared to normal watered plants (Fig. 1). It has been seen by other authors that CAT activity has increased in the leaves of drought-affected wheat plants (Simova-Stoilova et al., 2010). So, the fact proves that tolerant plants have more effective protection systems, which allow the cells and organs to function normally under stress. Increased CAT activity during water scarcity has been observed in barley leaves (Kublis, 2003), wheat (Luna et al; 2004), beans (Turkan et al., 2005) and grasses cultivated under mild climatic conditions (Fu and Huang, 2001). These results provide us with the fundamental evidence that ROS have increased leaf age.

It is known that APO is located in chloroplasts, mitochondria, microtelles and cytosol and is the main enzyme that utilizes hydrogen peroxide in plants. Ascorbate peroxidase is one of the enzymes that play a key role in the detoxification of hydrogen peroxide in the cell. In the research, APO activity was different from the CAT dynamics in terms of the dependence of leaf age. During aging, accompanied by drought stress, in the tolerant genotype Vugar, APO activity increased proportionally, reached its maximum (217 ± 7 μmol / mg protein min) on 35th day (May 21), and on the 42nd day (May 31), it again fell sharply (76 ±2 μmol/mg protein min) due to degradation processes at the end of aging.

However, 2 peaks are observed in APO activity during stressful senescence in the sensitive Tartar genotype. The first peak was recorded on the 21st day (May 7) and the second on the 35th day (May 21) as in the tolerant genotype. Thereby, the reduction of the leaf antioxidant status during aging is associated with lipid peroxidation, cell damage, and an increase in lipids, proteins, ROS oxidizing DNA, which results in cell death (Kukavica and Veljovic-Jovanovic, 2004). A great deal of information is available regarding the relationship of leaf aging with oxidative stress. The increase in plant age leads to increased oxidative stress, especially in chloroplasts (Munné-Bosch and Alegre, 2002). The biosynthesis and amount of ascorbic acid are reduced during the senescence process (Queval and Noctor, 2007; Srivalli and Khanna-Chopra, 2009).

These processes are also accompanied by decreased activity of antioxidant enzymes such as SOD, CAT, APO and pyridine nucleotides (Jiménez et al., 1998; Orendi et al., 2001; Srivalli et al., 2001). Increased ROS and decreased activity of antioxidant enzymes are observed in stress-induced senescence (Sandalio et al. 2001).

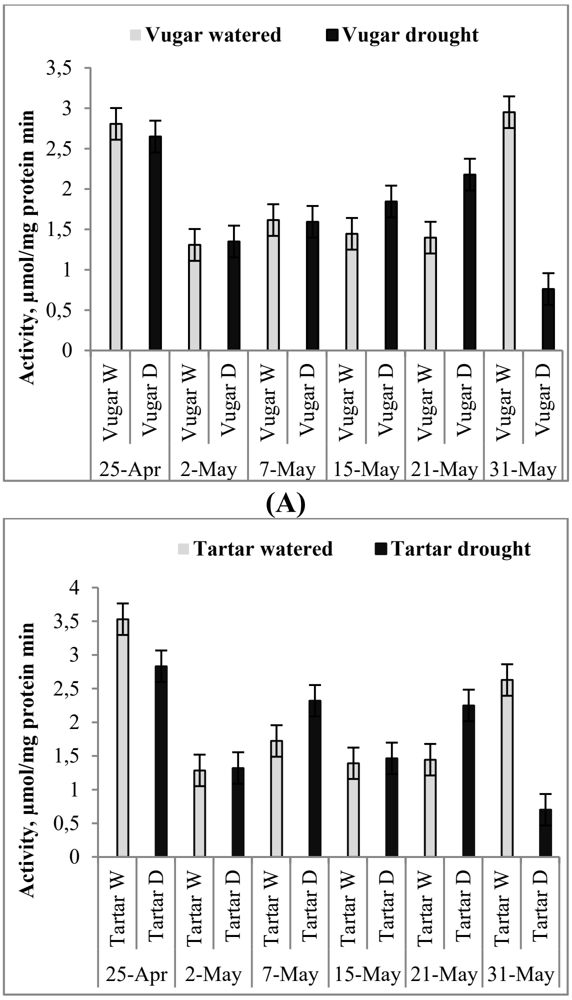


Fig. 2. Changes of APO enzyme activity during leaf senescence in wheat plants affected by drought stress (A-Vugar and B-Tartar).

When the expression of various genes that associate with aging is exposed to ozone and other effects, there has been an increase in the levels of ROS in older leaves (Miller et al., 1999; Navabpour et al,. 2003). Thus, the correlation between the key components of antioxidant defense systems, such as catalase and ascorbate peroxidase, and natural as well as drought-induced leaf senescence processes has been established.

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Quraqlıq stresinin təsirindən buğda bitkisində flaq yarpağın qocalması zamanı antioksidant fermentlərin fəallıqlarının dinamikası

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Buğda bitkisində fotosintez zamanı günəş enerjisinin udulmasında əsas rol oynayan və bitkinin məhsuldarlığını şərtləndirən flaq yarpağının fizioloji qocalması stres şəraitində yüksək məhsuldarlığın təmin edilməsi üçün vacib parametrlərdən biridir. Davamlılığına görə fərqlənən bərk buğda genotiplərində quraqlıq stresi və normal suvarma şəraitində flaq yarpağın qocalması zamanı askorbat peroksidaza (APO) və katalaza (KAT) fermentlərinin fəallığının dinamikası tədqiq edilmişdir. Flaq yarpağın əmələ gəlməsindən 7 gün sonra 6 nöqtədə ölçmələr aparılmışdır. Fermentlərin fəallığı yarpağın təbii və streslə müşayiət olunan qocalması zamanı fərqli olmuşdur. KAT-ın fəallığı həm normal suvarılan, həm də su qıtlığına məruz qalmış bitkilərin ən cavan və qocalmanın son dövründə olan yarpaqlarında nisbətən aşağı olmuşdur. KAT-ın maksimal fəallığı kontrol bitkilərdə 21 günlük flaq yarpaqlarında, quraqlığın təsirinə məruz qalan davamlı Vüqar genotipində 28 günlük, həssas Tərtər genotipində isə 21 günlük yarpaqlarda müşahidə edilmişdir. APO-nun fəallığı isə cavan yarpaqlarda daha yüksək olmuşdur. Kontrol bitkilərdə bu fermentin fəallığı qocalmanın tam sonunda yenidən yüksəldiyi halda, stresə məruz qalmış bitkilərdə qocalmanın daha sürətlə getməsi səbəbindən kəskin azalma müşahidə olunmuşdur. Həssas genotipdən fərqli olaraq, davamlı genotipdə yarpağın yaşı ilə APO-nun aktivliyi mütənasib artmışdır.

Açar sözlər: *Tritucum durum* Desf., *quraqlıq, flaq yarpağın qocalması, askorbat peroksidaza, katalaza*